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<110> University of Guelph

<120> Novel Inducible Genes From Alfalfa And Method Of Use Thereof

<130> 08-892370WO

<140> PCT/CA03/00964

<141> 2003-06-27

<150> 60/392,444

<151> 2002-06-28

<160> 19

<170> PatentIn version 3.1

<210> 1

<211> 474

<212> DNA

<213> *Medicago sativa*

<220>

<223> Nucleotide sequence of H7 coding region

<400> 1

atgggtgttt ttactttcaa ttagtgaacat gtctcaaccg tggctccagc taaaactctac 60

aaggctcttg caaaaagatgc ttagtgaatc gtcccaaagg tgatttctgc tgcccaaagt 120

gttgaaaattt ttgaaggaaa tggaggaccc ggaacttatta agaagctatc cattgttcaa 180

gatggcaaaa ccaactttgt gctacacaaa ttagattcag tggatgaggc aaactttgga 240

tataactaca gcttagtggg aggaacaggg ttggatgaaa gtttagagaa agttgaattt 300

gagacaaaaa ttgttgctgg ctctgatggt ggatccattt ttaagattt cgtaaataac 360

cataccaaag gtgtatcaac tctatctgaa gcagttacgtg aggagactaa ggccaaagga 420

actggactta tcaaggccat tgagggctac gtttagcaa accctaattt ctag 474

<210> 2

<211> 678

<212> DNA

<213> *Medicago sativa*

<220>

<223> Nucleotide sequence of H11 coding region

<400> 2

atggcctcca cactcagtct tgtcaagctt cccattcttt caagcatcaa gacacgccaa 60

tcAACCTCAA AACATGTTGT TCCACTTCCA TCCAAATTCA ATATTGTCCTC TCCCACCCCA 120
ctAAAGTTT CATTAGATCA TCAAATTAAAT ATCAAACAAA CTTCTTTCT ATCCCTCACA 180
gCAATCACAT TTCCATTCTT ATTGGATAACC AAAGAGTTG GGATATTGA AGGAAGAAC 240
TTTGTCTCA TTCACCCCAT TGTGTTGGGT GGTTTGTCT TCTATACTCT ATATGCTGGC 300
TATTTGGGGT GGCAATGGCG CGGAGTTAGG ACTATTCAAA ATGATATTAA TGAGCTCAAG 360
AAACAACCTCA AACCTGCACC GGTCGCCCT GATGGTAAAG CACTGAAAC TTCACCGCCA 420
TCACCTGTTG AACTTCAAAT CCAGAAACTT ACTGAGGAGA GGAAAGAGCT TATCAAAGGT 480
TCATACAGGG ATAAACACTT TAATGCTGGA TCCATACTTC TAGGATTGG TGTCTTTGAG 540
GCTGTTGGTG TGAGGACTCA ACACATGGTT AAGGACAGGA AAGCTATTTC CAGGTCCACA 600
TTTATTGCA GGAGCAGGCA TTACCGTCTT ATGGGCACTG GCAGCAGCTC TAGTACCAACC 660
GATGCAGAAA GGCAGTGA 678

<210> 3
<211> 744
<212> DNA
<213> *Medicago sativa*

<220>
<223> Nucleotide sequence of H12 coding region

<400> 3
atggcaacca acgaagatca aaagcaaact gaatctggaa gacatcaaga agttggtcac 60
aagagtcttt tacaaaagtga tgctcttac cagtatattc tagagaccag tgtcttccca 120
agagaacatg aagccatgaa agagttgaga gaggtcacag caaaacaccc atggaacatc 180
atgacaacct ctgcagatga aggacaattt ttgagcatgc tccttaaact tatcaatgct 240
aagaatacca tggaaattgg tgtctacact ggctactccc tccttgccac tgccctagct 300
attcctgaag atggaaagat tttggctatg gacattaaca aagaaaatta cgaattgggt 360
ctacctgtaa ttaaaaaaagc tggtggtgat cacaaaattg atttcagaga aggtccagct 420
cttccagttc ttgatgaaat gatcaaagac gaaaagaatc atggtagcta cgatttcatt 480
tttgtggatg ctgacaaaaga caattacctc aactaccata agaggttaat tgatcttggt 540
aaagtgggag gtgtgatcgg gtacgacaac accttatgga atggatctgt ggttgcaccc 600
cctgatgctc cattgaggaa gtatgttagg tactatagag atttgtttt ggagcttaac 660
aaggcttgg ctgtggaccc taggattgaa atatgtatgc ttccctgttgg tgatggaatc 720

actatctgcc gtaggatcaa gtaa

744

<210> 4
<211> 634
<212> DNA
<213> *Medicago sativa*

<220>
<223> Nucleotide sequence of H7 regulatory region

<400> 4
acgcgtggtc gacggcccg gctggtaact aagtattact attaccaaat ttttaggacc 60
ccacccatga caccattgct atatttcaat ttggggaaaat attgctataa agttactgta 120
gtaactttta gaagaaggtt tttttttaa ggattttaga ggaaggtag caacacacat 180
gcactttaaa tatacatttt ttcttataaa gttttgtat cgagttgaga aatcatatat 240
atactcataaa atcatgtgga tttcatataa tttaatagaa cacataaatt ttaaccgaga 300
aataaaagtgt tgcaaataata tgtaaaaaga gtacgtttagt aacattattt taatttcttt 360
tattcaatcc acactttgag tcatggactg ctataactat tcattttgtt tttcgcaacc 420
taatttagaga ttgtccagat acaaagagga gtaacctaataaataatataaaatattc 480
accaacggcc tcagtaagct acttgagcta aacaatgaga tttccaaataa aggttaggtcc 540
ttcccaagtt ctataaataag catccctcac catgtcataa accgcacac aagttatata 600
ctgttattcat actatacact tattttca tttta 634

<210> 5
<211> 438
<212> DNA
<213> *Medicago sativa*

<220>
<223> Nucleotide sequence of H11 regulatory region

<220>
<221> misc_feature
<222> (1)..(438)
<223> where "n" is a or g or c or t or other

<400> 5
cagaaccccg anaggctggt gctagtatgg cttcggttta atacgactca ctatagggcg 60
cgcggtggtcg acggcccggtt ctggtaatcgat cgagtaacga ttcatcatat ctacactag 120
ggatgaatga tttattatttg agtttatgaa tttgaactat tacttctaata ttctaaatga 180

agacatttaa gtaaaagatt aaaatattct agtttcaa attttgatt ttagaattta 240
aatttaatct taaaaaaaaa attaaattta aagaagataa aaagggagaa aataaataga 300
tgaatataat ttgtaaacat gaagaccta tctccagtaa aaaaacatat ggaccttatac 360
ttttgaggt aggaaggatc tacgcgggaa acctcttcct gactgtgaac cccgtatgca 420
gaggcagaga cagagagt 438

<210> 6
<211> 936
<212> DNA
<213> *Medicago sativa*

<220>
<223> Nucleotide sequence of H12 regulatory region

<400> 6
aaatacaaag gtgaccttat ttgcaaata atccatgcat ggaaatgcata ctttttg 60
aaaatgggtt tatctgaatt cttaatgttac gtgaaaattt aatacatttc attttagata 120
aatttattat taaaattcac acttagatgg cctaaaaattt aacacttatt ttacaattt 180
caaataaaat atacgacgaa atgagtgtaa tttagtttgtt taagcatcgtt caaagcttgg 240
agagaaagat catagttga tctttgaaaa ctatactatt gaaaagggtt aagatattctt 300
acctccaaca aaatttattt gatagtcgat tcaaatttac aaaatttggaa aaatattttg 360
taaattgtta agttggaaa aatatgttaa ttcaattt accatttgcata cattttctta 420
atctcaaattt acatttaagg gatgttactt acttcgtttt tgtacaattt tttacaattt 480
taacattttt aaaaatgtgtt ttggtagata aaaaatgtgtt gtattcttta taagagattt 540
tgttttctt ttgttttaac ttataaaaata aatatataatt ttatattttt ttaacgttag 600
attgttaagaa ttcatattataa gattatgtca ttccctcaaa agaaaattttt atgtatgtcat 660
tttcataactt cattttctat aaatacagaa aatcctcaaa aatgaaaaac ctcggtcaaa 720
aaataaaaaga aaaaacatcaa tagtggactg gcccacactc attgctttgc tttagttaga 780
gaaagtagac ctcaccaacc acgaaccgga cggccgaccgg ttcaacccaaa catcacacca 840
attttcccaa accataccgg ttcccttc ctttatataa ccattccttc ccctttctc 900
taaccaagct tcattcaactt cttcaacacata tatcag 936

<210> 7
<211> 1424

<212> DNA
<213> *Medicago sativa*

<220>
<223> Nucleotide sequence of genomic H7

<400> 7
acgcgtggtc gacggcccg gctggacta aagtattact attaccaaat ttttaggacc 60
ccacccatga caccattgct atattcaat ttggaaaat attgctataa agttaactgta 120
gtaacttttta gaagaaggtt tttttttaa ggattttaga ggaaggtag caacacacat 180
gcactttaaa tatacatttt ttcttataaa gttttgtat cgagttgaga aatcatatat 240
atactcataa atcatgtgga tttcatataa ttaatagaa cacataaatt ttaaccgaga 300
aataaaagtgt tgcaaataata tgtaaaaaga gtacgttgtt aacattattt taatttctt 360
tattcaatcc acactttgag tcatggactg ctatactaatt tcattttgtt tttcgcaacc 420
taatttagaga ttgtccagat acaaagagga gtaacctaatt aaataaaatataaaatattc 480
accaacggcc tcagtaagct acttgagcta aacaatgaga tttccaaataa aggttaggtcc 540
ttcccaagtt ctataaaatag catccctcac catgtcataa accgcacac aagttatata 600
ctgtattcat actatacact tatcctttca tttacttctt gcatattgtat cttgttatac 660
ttgatataata tatacatgggt gttttactt tcaatgatga acatgtctca accgtggctc 720
cagctaaact ctacaaggct cttgcaaaag atgctgatga aatcgcccc aaggtgattt 780
ctgctgcccc aagtgttgaa attgttgaa gaaatggagg accccgaact attaagaagc 840
tatccattgt tgaagatggc aaaaccaact ttgtgctaca caaatttagat tcagtggatg 900
aggcaaactt tggatataac tacagcttag tgggaggaac agggttggat gaaagtttag 960
agaaagttga atttgagaca aaaattgttg ctggctctga tgggtggatcc attgttaaga 1020
tttcagtgaa ataccataacc aaaggtgatg caactctatc tgaagcagta cgtgaggaga 1080
ctaaggccaa aggaactgga cttatcaagg ccattgaggg ctacgtttta gcaaacccta 1140
attactagcc aattaaaccc tattgaggac tttaatttg gttgtgttgc ttcatgcgaa 1200
taataattaa agtttatgat gcggttgaag tgggttgagt atacatcaag gtctttggct 1260
cgtacatgtg tgggtggctt gttggatgtt gtgaggtttg agtgcatttt tgggtgttta 1320
aaaacaaaaaa cctatgttgc gttgggtgata aggtttgca ccacatgtat tatgcaataa 1380
ataatgcaaa agaattttat cgcgaaaaaa aaaaaaaaaaaa aaaa 1442

<210> 8
 <211> 1482
 <212> DNA
 <213> *Medicago sativa*

<220>
 <223> Nucleotide sequence of genomic H11

<220>
 <221> misc_feature
 <222> (1)...(1482)
 <223> Where n is a or g or c or t or other

<400> 8

| | |
|-------------------------|------|
| cagaaccccg anaggctgg | 60 |
| gctagtatgg cttcggttga | |
| atacgactca ctataggcg | |
| cgcgtggtcg acggccggg | 120 |
| ctggtatcatcg cgagtaacga | |
| ttcatcatat ctcacactag | |
| ggatgaatga tttattattg | 180 |
| agtttatgaa tttgaactat | |
| tacttcta at ttcataatga | |
| agacatttaa gtaaaagatt | 240 |
| aaaatattct agtttcaa | |
| at atttggatt ttagaattt | |
| aatttaatct ttaaaaaaaa | 300 |
| attaaattta aagaagataa | |
| aaaggagaa aataaataga | |
| tgaatataat ttgtaaacat | 360 |
| gaagaccta tctccagtaa | |
| aaaaacatat ggaccttata | |
| ttttgaggt aggaaggatc | 420 |
| tacgcgggaa acctttcct | |
| gactgtgaac cccgtatgca | |
| gaggcagaga cagagagtat | 480 |
| gcctccaca ctcagtc | |
| tcaagcttccattttca | |
| agcatcaaga cacgccaatc | 540 |
| aacctcaaaa catgtgttc | |
| cacttccatc caaattcaat | |
| attgtccctc ccacccact | 600 |
| aaagtttca ttagatcatc | |
| aaattaatata caaacaact | |
| tctcttctat ccctcacagc | 660 |
| aatcacatccatccattt | |
| tggtatccaa ggcaagcaag | |
| caagcaagca tcctattcta | 720 |
| ttctattctt tcatccatata | |
| ctttactttt ttgtttctat | |
| accaatccat gatatgaatg | 780 |
| ttgttgaaac aggatgcact | |
| tgctgttggt ggagagttt | |
| ggatatttga aggaagaaca | 840 |
| tttgctctca ttcacccat | |
| tgtgttggtt ggtttgttct | |
| tctatactct atatgctggc | 900 |
| tatgggggtt ggcaatggcg | |
| ccgagtttagg actattcaaa | |
| atgatattaa tgagctcaag | 960 |
| aaacaactca aacctgcacc | |
| ggtcgcctt gatggtaaag | |
| cacttgaac ttcacccgcca | 1020 |
| tcacctgttg aacttcaat | |
| ccagaaactt actgaggaga | |
| ggaaagagct tatcaaaggt | 1080 |
| tcatacaggg ataaacactt | |
| taatgctgga tccatacttc | |
| taggatttgg tgtcttgag | 1140 |
| gctgttggt tgaggactca | |
| acacatggtt aaggacagga | |
| aagctatttc caggtccaca | 1200 |
| tttatttgcg ggaggcaggca | |
| ttaccgtctt atgggcactg | |

gcagcagctc tagtaccacc gatgcagaaa ggcagtgaaa cagccagaaa tcttcacatt 1260
gctctgaata cattgaatgt tcttctttt gtgtggcaga ttcccactgg acttgatatt 1320
gtatggaaag tgttttagtt cacaaaatgg ctttgaatgt atgattctca tatgtaaagta 1380
agttcccagg tattttactt tcaaattcagt atttggcaat atcaataaaat gcaaaaatttg 1440
ctattctgca ttttcaaaaa aaaaaaaaaaa aaaaaaaaaaa aa 1482

<210> 9
<211> 1906
<212> DNA
<213> *Medicago sativa*

<220>
<223> Nucleotide sequence of genomic H12

<400> 9
aaatacaaaag gtgaccttat tttgcaaaata atccatgcat ggaaatgcat catccttttg 60
aaaatgggtt tatctgaatt cttaagttac gtgaaaattt aatacatttc attttagata 120
aatttattat taaaattcac acttagatgg cctaaaaattt aacacttatt tttaacaatt 180
caaataaaaat atacgacgaa atgagtgtaa ttttagttggta aagcatcgt caaagcttgg 240
agagaaaagat catagttga tctttgaaaa ctatactatt gaaaagggtg aagatatcta 300
acctccaaca aaatttattt gatagtcgtat tcaaatttac aaaatttggaa aaatattttg 360
taaattgtta agttggaaaa aatatgttaa ttttcaaattt accatttgcata cattttctta 420
atctcaaattc acatttaagg gatgttgact acttcgttt tgtacaattc ttacaattt 480
taacatttat aaaatgtgtt ttggtagata aaaagtgtga gtattcttta taagagattt 540
tgttttctt ttgttttaac ttataaaaata aatatattttt ttatattttt ttaacgttag 600
attgtaaagaa ttcattataa gattatgtca ttccctcaaa agaaaatttag atgatgtcat 660
tttcataact cattttctat aaatacagaa aatcctcaaa aatgaaaaac ctccgtcaaa 720
aaataaaaaga aaaacatcaa tagtggactg gcccacactc attgctttgc ttttagtatga 780
gaaagttagac ctcaccaacc acgaaccgga cgccgaccgg ttcaacccaaa catcacacca 840
atttcctaa accataccgg ttttccctc ctttatataa ccattcctctc ccctcttctc 900
taaccaagct tcattcaact cttcaacaca tatcagaaaac agaaaaaaga agcaaaaacat 960
tccaagaatt taacaatggc aaccaacgaa gatcaaaaagc aaactgaatc tggaaagacat 1020
caagaagttg gtcacaaagag tctttacaa agtgtatgctc tttaccagta tattctagag 1080

<210> 10

E2113 157

<211> 157

<213> *Medicago sativa*

52203

<223> Amino acid sequence encoded by H7 coding region

<400> 10

Met Gly Val Phe Thr Phe Asn Asp Glu His Val Ser Thr Val Ala Pro
1 5 10 15

Ala Lys Leu Tyr Lys Ala Leu Ala Lys Asp Ala Asp Glu Ile Val Pro
 20 25 30

Lys Val Ile Ser Ala Ala Gln Ser Val Glu Ile Val Glu Gly Asn Gly
35 40 45

Gly Pro Gly Thr Ile Lys Lys Leu Ser Ile Val Glu Asp Gly Lys Thr
50 55 60

Asn Phe Val Leu His Lys Leu Asp Ser Val Asp Glu Ala Asn Phe Gly
65 70 75 80

Tyr Asn Tyr Ser Leu Val Gly Gly Thr Gly Leu Asp Glu Ser Leu Glu
85 90 95

Lys Val Glu Phe Glu Thr Lys Ile Val Ala Gly Ser Asp Gly Gly Ser
100 105 110

Ile Val Lys Ile Ser Val Lys Tyr His Thr Lys Gly Asp Ala Thr Leu
115 120 125

Ser Glu Ala Val Arg Glu Glu Thr Lys Ala Lys Gly Thr Gly Leu Ile
130 135 140

Lys Ala Ile Glu Gly Tyr Val Leu Ala Asn Pro Asn Tyr
145 150 155

<210> 11

<211> 247

<212> PRT

<213> *Medicago sativa*

<220>

<223> Amino acid sequence encoded by H12 coding region

<400> 11

Met Ala Thr Asn Glu Asp Gln Lys Gln Thr Glu Ser Gly Arg His Gln
1 5 10 15

Glu Val Gly His Lys Ser Leu Leu Gln Ser Asp Ala Leu Tyr Gln Tyr
20 25 30

Ile Leu Glu Thr Ser Val Phe Pro Arg Glu His Glu Ala Met Lys Glu
35 40 45

Leu Arg Glu Val Thr Ala Lys His Pro Trp Asn Ile Met Thr Thr Ser
50 55 60

Ala Asp Glu Gly Gln Phe Leu Ser Met Leu Leu Lys Leu Ile Asn Ala
65 70 75 80

Lys Asn Thr Met Glu Ile Gly Val Tyr Thr Gly Tyr Ser Leu Leu Ala
85 90 95

Thr Ala Leu Ala Ile Pro Glu Asp Gly Lys Ile Leu Ala Met Asp Ile
100 105 110

Asn Lys Glu Asn Tyr Glu Leu Gly Leu Pro Val Ile Lys Lys Ala Gly
115 120 125

Val Asp His Lys Ile Asp Phe Arg Glu Gly Pro Ala Leu Pro Val Leu
130 135 140

Asp Glu Met Ile Lys Asp Glu Lys Asn His Gly Ser Tyr Asp Phe Ile
145 150 155 160

Phe Val Asp Ala Asp Lys Asp Asn Tyr Leu Asn Tyr His Lys Arg Leu
165 170 175

Ile Asp Leu Val Lys Val Gly Val Ile Gly Tyr Asp Asn Thr Leu
180 185 190

Trp Asn Gly Ser Val Val Ala Pro Pro Asp Ala Pro Leu Arg Lys Tyr
195 200 205

Val Arg Tyr Tyr Arg Asp Phe Val Leu Glu Leu Asn Lys Ala Leu Ala
210 215 220

Val Asp Pro Arg Ile Glu Ile Cys Met Leu Pro Val Gly Asp Gly Ile
225 230 235 240

Thr Ile Cys Arg Arg Ile Lys
245

<210> 12

<211> 44

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence of PCR-Select cDNA synthesis primer; see Fig.2

<220>

<221> misc_feature

<222> (1)..(44)

<223> where n is a or g or c or t or other

<400> 12

ttttgtacaa gctttttttt tttttttttt tttttttttt ttnn

44

<210> 13

<211> 44

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence of Adaptor 1; see Fig.2

<400> 13

ctaatacgtac tcactatagg gctcgagcgg ccgccccggc aggt

44

<210> 14

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence of Adaptor 2R; see Fig.2

<400> 14

ctaatacgtac tcactatagg gcagcggtggt cgccggccgag gt

42

<210> 15

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence of PCR primer 1; see Fig.2

<400> 15

ctaatacgtac tcactatagg gc

22

<210> 16

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence of nested PCR primer 1; see Fig.2

<400> 16

tcgagcgccc gcccgggca

19

<210> 17

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence of nested PCR primer 2R; see Fig.2

<400> 17

agcgtggtcg cggccgaggt

20

<210> 18

<211> 10

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence of complement (partial); see Fig.2

<400> 18

ggcccggtcca

10

<210> 19

<211> 10

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence of complement (partial); see Fig.2

<400> 19

gccggctcca

10